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Cutaneous Mast Cell Maturation Does Not Depend on an Intact Bone Marrow Microenvironment

MICHAEL R. CHARLEY, M.D., ANWAR MIKHAEL, M.D., RICHARD D. SONTHEIMER, M.D., JAMES N. GILLIAM, M.D. AND MICHAEL BENNETT, M.D.

Departments of Dermatology and Pathology, University of Texas Health Science Center at Dallas, Dallas, Texas, U.S.A.

We sought to determine whether the maturation of murine cutaneous mast cells from stem cells depends on an intact bone marrow microenvironment. Normal bone marrow cells (+/+) were infused into 2 groups of mast cell-deficient mice: WBB6F$_1$-W/W^v mice and ^{89}Sr-pretreated W/W^v mice. ^{89}Sr is a long-lived bone-seeking radioisotope which provides continuous irradiation of the marrow and thereby ablates the marrow microenvironment.

Skin biopsies revealed that the ^{89}Sr-pretreated mice and the controls had repopulated their skin with mast cells equally well. Natural killer cell function was significantly depressed in the ^{89}Sr-treated mice, confirming that the marrow microenvironment had been functionally altered. We conclude that, although the precursors for cutaneous mast cells are marrow derived, they do not need an intact marrow microenvironment for maturation.

The concept of cell maturation being dependent on tissue-specific microenvironments is well established, but poorly understood. In the hemopoietic system, Wolf and Trentin demonstrated inductive microenvironments in the marrow and spleen which determined erythropoietic vs granulopoietic differentiation of colony-forming stem cells [1]. The thymus and bursa of Fabricius are the classical central lymphoid organs necessary for differentiation of T and B cells, respectively [2]. Abnormal marrow fibroblastoid cells in op/op mice are associated with reduced macrophage numbers and osteopetrosis, due to a block in monocyte/osteoclast maturation [3]. Natural killer (NK) cell development to full functional capacity is dependent on an intact marrow microenvironment. Osteopetrosis (in mi/mi mice, or induced by estradiol) results in defective NK cell activity as does treatment of mice with the long-lived, bone-seeking radioisotope ^{89}Sr [4,5].

A skin microenvironment has been shown to be necessary for cutaneous mast cell development. WCB6F$_1$-S1/S1^d mice are the prototypes. Their skin will not support mast cell development when grafted to normal mice, but their stem cells can give rise to mast cells when transferred into other mast cell-deficient mice [6,7].

The recent demonstration of high-affinity Fc receptors for IgE on an NK cell line [8] raised the possibility that cutaneous mast cells and NK cells were related. To test the hypothesis that cutaneous mast cells resembled NK cells in needing an intact marrow microenvironment, we utilized two tools: (1) mice that were deficient in cutaneous mast cells due to a stem cell defect (WBB6F$_1$-W/W^v), and (2) a bone marrow ablation technique using ^{89}Sr. We observed that normal marrow (+/+) reconstituted both ^{89}Sr-pretreated and control W/W^v mice with cutaneous mast cells equally well. We conclude that cutaneous mast cells do not need an intact bone marrow microenvironment for maturation.

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Reprint requests to: Michael R. Charley, M.D., Southwestern Medical School, The University of Texas Health Science Center at Dallas, 5323 Harry Hines Boulevard, Dallas, Texas 75235.

Abbreviations:
DNFB: dinitrofluorobenzene
NK: natural killer

MATERIALS AND METHODS

Animals

Female (WBB6F$_1$-W/W^v and +/+) mice congenic at the dominant spotting locus were purchased from the Jackson Laboratory, Bar Harbour, Maine. W/W^v mice are deficient in cutaneous mast cells due to a stem cell defect; +/+ mice are their normal, mast cell replete, littermates [9].

Treatment with ^{89}Sr

^{89}Sr Cl$_3$ was purchased from Oak Ridge National Laboratory, Oak Ridge, Tennessee. Each mouse was injected i.p. with 75 μCi.

Bone Marrow Transplantation

Three and 10 days after injection of ^{89}Sr, or of medium alone (control group), each mouse was infused with 20×10^6 +/+ bone marrow cells. The marrow cells were obtained by flushing the long bones of the legs with cold Hanks' balanced salt solution containing heparin (2 units/ml), penicillin (50 units/ml) and streptomycin (50 μg/ml). A monodispersed suspension was prepared by repeated aspiration through an 18-gauge needle. The cells were washed, enumerated with a hemocytometer, and adjusted to 40×10^6 viable cells/ml. The cells were infused into a lateral tail vein.

Elicitation of Cutaneous Mast Cells

Dinitrofluorobenzene (DNFB) (0.5% in olive oil and acetone) was applied to the shaved abdomen of mice to stimulate mast cell accumulation 5 weeks after the second infusion of bone marrow cells.

Histology

The skin was biopsied 2 weeks after applying the DNFB. The skin was fixed in 10% buffered formalin and 4 μm-thick sections were stained with acidified toluidine blue. The numbers of mast cells per high-powered field (14 per mouse) were determined.

Natural Killer Cell Assay

Recipient mice were injected with 0 or 100 μg polyinosinic: polycytidylic acid i.p. 1 day before harvesting spleen cells. This agent augments NK effector cell function. To obtain spleen cells, the spleens were flushed with RPMI 1640 medium while being compressed with blunt forceps. The cells were dispersed by aspiration through an 18-gauge needle and particulate matter was removed by passing the suspension over 100 mesh/inch stainless steel wire gauze. The cells were washed and resuspended in RPMI 1640 containing 10% fetal calf serum and antibiotics. After counting the cells, they were adjusted to 1×10^7/ml. YAC-1 lymphoma cells of A-strain origin were maintained in tissue culture. These cells were washed in RPMI 1640 medium and the cells (6×10^6) were incubated with 100 μCi $Na_2{}^{51}CrO_4$ (New England Nuclear Corporation, Boston, Massachusetts) for 90 min at 37°C in medium not containing serum. The cells were washed 3 times and were resuspended in medium with 10% fetal calf serum. The cells were counted and adjusted to 2×10^5 viable cells/ml. Round-bottomed wells of microtiter plates (Costar, Cambridge, Massachusetts) were plated with 2×10^{14} ^{51}Cr-labeled YAC-1 cells in 0.1 ml. Effector spleen cells were added (triplicate samples, in numbers 10^6 to 2.5×10^5) in 0.1 ml to obtain effector:target cell ratios of 50:1, 37.5:1, 25:1, and 12.5:1. Control wells contained only target cells (spontaneous release values)

or 0.025 ml 2% saponin solution (maximum release values). The cells were incubated at 37°C in a 5% CO_2/air humidified atmosphere for 4 h. The plate was centrifuged at 50 *g* for 5 min to pellet the cells. Volumes of 0.1 ml supernatant fluids were removed and the ^{51}Cr radioactivity was measured in a Packard PRIAS gamma scintillation counter. The formula for calculating cytotoxicity is:

$$\%\ \text{specific cytotoxicity} = \frac{^{51}\text{Cr cpm experimental} - \text{spontaneous release}}{\text{maximum} - \text{spontaneous release}} \times 100$$

RESULTS

Mast Cell Accumulation in Skin

The application of DNFB, which is both irritating and sensitizing immunologically, increases the numbers of mast cells in the dermis of normal mice and reduces the variability in numbers of mast cells per high-powered field. W/W^v mice that were neither treated with ^{89}Sr nor infused with +/+ bone marrow cells, did not have significant mast cell numbers, even after application of DNFB (Table I). DNFB did stimulate the accumulation of dermal mast cells in W/W^v mice repopulated with +/+ marrow cells. Pretreatment with ^{89}Sr to destroy the marrow microenvironment did not prevent the generation of cutaneous mast cells (Table I).

Natural Killer Cell Function

The ability of spleen cells from the chimeric mice to lyse YAC-1 target cells was stimulated significantly by polyinosinic:polycytidylic acid, an interferon inducer (Fig 1). ^{89}Sr-treated W/W^v recipients of the marrow cells were relatively depleted of NK cell activity, as expected [11]. Although we did not examine the marrow of the actual mice used in this experiment, the low level of NK activity is as expected for nearly complete ablation of the marrow microenvironment.

DISCUSSION

The data presented here indicate that cutaneous mast cells are not marrow-dependent. ^{89}Sr-treated, mast cell-deficient W/W^v mice repopulated their skin with mast cells following infusion of normal bone marrow, equally as well as controls. ^{89}Sr destroys the marrow, and the spleen becomes the sole source of myelopoietic stem cells [10]. These stem cells give rise to erythropoietic cells, granulopoietic cells, and megakaryocytes. Moreover, B-cell, T-cell, and macrophage functions are intact in mice treated with ^{89}Sr [5]. So far, only NK cells and effectors for marrow allograft rejection have been shown to be marrow dependent [11,12].

Although mast cells have been shown to be unnecessary for the delayed in time reaction of contact hypersensitivity [13], it was of interest to find that DNFB could increase the number of dermal mast cells. We did not look at different concentrations of DNFB, and initially chose the irritating dose of 0.5% because of published use of another irritant, methylcholanthrene [14] for stimulating mast cells. We doubt that this concentration of 0.5% is optimal because it causes focal epidermal necrosis and dermal granulation tissue, making large areas unsuitable for counting mast cells. We did not look at mucosal mast cell counts. There have been recent reports [15] that connective tissue mast cells and mucosal mast cells may behave differently. Therefore, it is important not to generalize the conclusions from our skin mast cell data.

TABLE I. *Cutaneous mast cells in W/W^v mice repopulated with +/+ bone marrow cells*

Mouse genotype	*n*	Pretreatment of mice[a]	Application of DNFB[b]	Mast cells/hpf Mean ± SD[c]
+/+	3	None	−	7.6 ± 2.5
W/W^v	3	None	−	Rare
W/W^v	3	None	+	Rare
W/W^v	1	+/+ BMC	−	1.4 ± 3.3
W/W^v	3	+/+ BMC	+	17.0 ± 9.0
W/W^v	3	^{89}Sr, +/+ BMC	+	18.0 ± 12.0

[a] +/+ BMC = +/+ bone marrow cells; ^{89}Sr = mice were injected i.p. with 75 μCi ^{89}Sr; +/+ bone marrow cells (BMC), (2×10^7) were infused 3 and 10 days later.

[b] 0.5% dinitrofluorobenzene (DNFB) in olive oil and acetone were applied to shaved skin 5 weeks after cell transfer.

[c] Sections were stained with toluidine blue; 14 high-powered fields (hpf) were examined per specimen. Mean values of ^{89}Sr vs no ^{89}Sr were not significantly different $p > 0.5$.

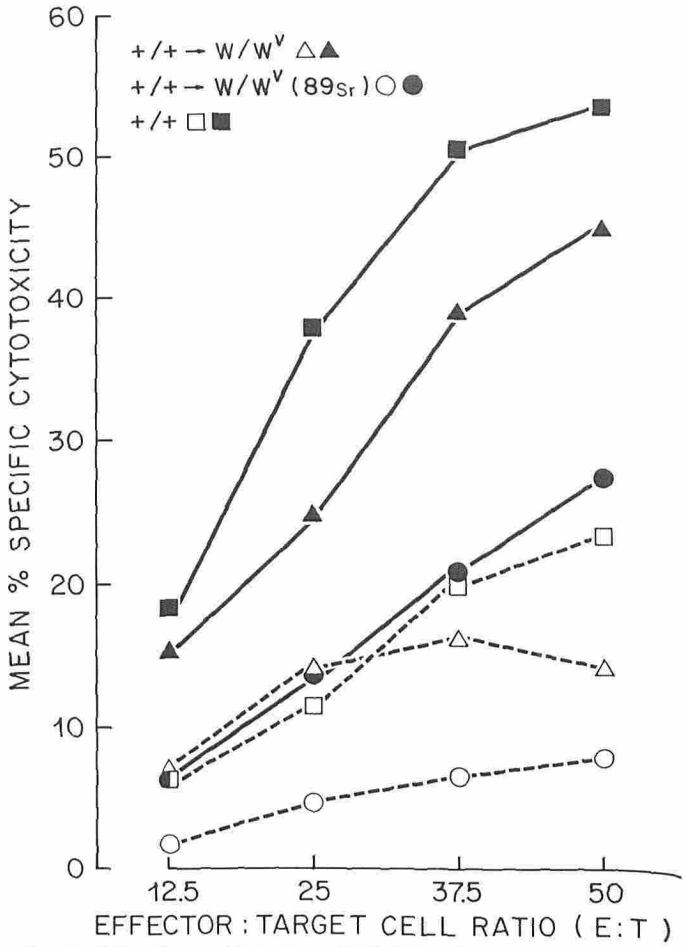

FIG 1. NK cell-specific lysis of YAC-1 lymphoma cells. *Open symbols* represent mice not boosted with polyinosinic:polycytidylic acid (poly I:C); *solid symbols* represent mice treated with poly I:C.

The ^{89}Sr effect on NK cell activity was not as marked in this experiment as it has been in those using other murine strains. The primary reason is that WBB6F$_1$ mice have low NK cell activity levels [16]. Demonstration of decreases in activity, was therefore limited (Fig 1). Nevertheless, ^{89}Sr is known to reliably cause severe damage to the marrow tissue [10].

In summary, the repopulation of skin mast cells in W/W^v mice by +/+ marrow cells was not influenced by previous treatment with ^{89}Sr to destroy the marrow microenvironment. Thus, cutaneous mast cells, although marrow-derived, do not resemble NK cells in needing an intact marrow microenvironment for maturation.

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